Bull. Environ. Contam. Toxicol. (2004) 73:832–837

DOI: 10.1007/s00128-004-0502-1

Bulletin of Environmental Contamination and Toxicology

Monitoring of DDT Residues in Bovine Milk in Punjab, India

H. K. Cheema, B. K. Kang, B. Singh

Department of Entomology, Punjab Agricultural University, Ludhiana 141 004, India

Received: 16 January 2004/Accepted: 30 July 2004

Milk has long been recognized as the most wholesome and complete single food available in nature for health and as a medicine, both preventive and curative. It is a complete fluid comprising of all nutrients required by a neonate for growth and sustenance (Mathur *et al.* 2000). India contributes 13 per cent of world's milk production. Thus it is important to monitor its quality and safety standards especially for export purposes.

The organochlorine insecticides are the most persistent compounds and are either banned or severely restricted for use in most developed countries in the world. However, DDT constituted the bulk of pesticides consumed in India for about five decades. Dairy milk and its products in particular have been shown to have a high incidence of contamination with residues of persistent organochlorine insecticides in India (Gupta *et al.* 1997; Kalra *et al.* 1999). However, analysis of samples of milk collected from Ludhiana during last 3-4 years revealed that the residues of this insecticide have started declining which could be due to the ban imposed on the insecticide for use in agriculture. Keeping this in view, the present investigations were undertaken to have a wider picture on the scenario of DDT contamination of milk in Punjab.

MATERIALS AND METHODS

Three samples of milk were collected every month from The Punjab State Cooperative Milk Producers Federation (MILKFED) depots of nine Milk Plants situated at Amritsar, Bathinda, Gurdaspur, Hoshiarpur, Jalandhar, Ludhiana, Mohali, Patiala, and Sangrur in Punjab. The collection period accounted for one year, with a total of 324 milk samples. Each sample consisted of 500 mL of pasteurized milk with 4.5 per cent fat content packed in a sealed polyethylene pouch. The samples were transported to the laboratory under low temperature conditions and processed on the same day.

All the solvents were glass distilled before use. All other chemicals were of analytical grade. The suitability of the reagents/ solvents was further checked by running reagent blanks.

Correspondence to: H. K. Cheema, c/o: B. Singh

Reference pesticide standards used were p,p'- DDE (p,p'- bis (chlorophenyl)- 1, 1 dichloroethane), p,p'- TDE (p,p'- bis (chlorophenyl)- 1, 1, 1, 1, 2- tetrachloroethane), p,p'- DDT (p,p'- bis (chlorophenyl)- 1, 1, 1- trichloroethane), o,p'- DDE (o,p'- bis (chlorophenyl)- 1, 1- dichloroethene), o,p'- TDE (o,p'- bis (chlorophenyl)- 1, 1, 1, 2- tetrachloroethane) and o,p'- DDT (o,p'- bis (chlorophenyl)- 1, 1, 1-trichloroethane) all > 95 % pure, were obtained from U.S. Environmental Protection Agency, North Carolina, U.S.A.

The extraction and cleanup of the milk was done by following the method of Battu *et al.* (2003). Five mL of milk sample was thoroughly mixed with 20 g pre-washed and activated silica gel and 20 g anhydrous sodium sulphate to form a free flowing powder. This powdered sample was made into slurry and packed quantitatively along with 50 mL dichloromethane into an extraction glass column (60 cm x 2 cm i. d.) containing about 40 mL dichloromethane over a plug of cotton. Care was taken that no air bubble was trapped inside the column. The column was stoppered and left for about 90 minutes. Dichloromethane was eluted drop wise from the column. When dichloromethane was about to reach the level of the adsorbent, the column was re-eluted with 150 mL mixture of acetone: dichloromethane (2:1, v/v). A pinch of anhydrous sodium sulphate was added to the eluate to remove turbidity, if any. The eluate was concentrated to about 5 mL on a rotary vacuum evaporator to ensure complete removal of dichloromethane and the final volume was made to about 10 mL with acetone in a graduated stoppered centrifugation tube.

The residues of pesticides in the cleaned up extract were quantified on a gas-liquid chromatograph (GLC) equipped with ^{63}Ni electron capture detector (ECD). A Pyrex glass columns (2m x 2 mm id) packed with ready to use 1.5 per cent SP-2250 + 1.95 per cent SP2401 on Supelcoport (80-100 mesh) was used for the analysis of DDT and its analogues. The standardized operating conditions were as follows: column temperature 220°C; injection port temperature 250°C and detector temperatures 280°C and carrier gas nitrogen flow rate 40 mL min^{-1}. The minimum limit of determination was 0.01 μg g^{-1} for the compounds analyzed. The recoveries of the DDT and related compounds spiked at levels ranging from 0.1 to 1.0 μg g^{-1} were more than 83 per cent. Therefore, the results are presented as such without applying correction factor.

RESULTS AND DISCUSSION

Out of 324 milk samples analyzed 86 samples revealed the presence of DDT residues which were present in the form of o,p'-DDE, p,p'-DDE, o,p'-DDT, p,p'-DDD and p, p'- DDT. Out of these, eight samples were found to exceed the prescribed limit of 0.05 of mg kg^{-1} (whole milk basis). Twelve (33.33 per cent) samples from Amritsar contained total DDT residues with average and range as 0.01 mg kg^{-1} and BDL – 0.05 mg kg^{-1} respectively (Table 1). DDT residues were present in the form of o,p'-DDE, p,p'-DDE, o,p'-DDT and p,p'-DDT. However, fourteen samples from Gurdaspur contained total DDT residues ranging from BDL – 0.05 mg kg^{-1} but no sample exceeded the erl. DDT residues were present in the

Table 1. Concentration of ∑-DDT in bovine milk in Punjab.

Location	Number of samples		Mean (mg kg^{-1})	Range (mg kg^{-1})	No. of samples > erl (%)
	analyzed	contaminated(%)			
Amritsar	36	12 (33.33)	0.01	BDL- 0.05	1 (2.77)
Gurdaspur	36	14 (38.89)	0.01	BDL - 0.05	NIL
Hoshiarpur	36	9 (25.00)	0.01	BDL - 0.18	1 (2.77)
Jalandhar	36	8 (22.22)	0.01	BDL - 0.13	3 (8.33)
Ludhiana	36	4 (11.11)	BDL	BDL - 0.03	NIL
Mohali	36	9 (25.00)	0.01	BDL - 0.21	1 (2.77)
Patiala	36	10 (27.77)	0.01	BDL - 0.05	NIL
Sangrur	36	8 (22.22)	0.01	BDL - 0.13	1 (2.77)
Bathinda	36	12 (33.33)	0.01	BDL - 0.06	1 (2.77)

∑-DDT represents o,p'-DDE, p,p'-DDE, o,p'-DDT, p,p'-DDD and p,p'-DDT
BDL (Below Detectable Limit) < 0.01 mg kg^{-1}
Extraneous residue limit (erl)= 0.05 mg kg^{-1} as per Codex Maximum Residue Limits, FAO 1999.
The residue values are calculated on whole milk basis with fat content of 4.5 per cent.

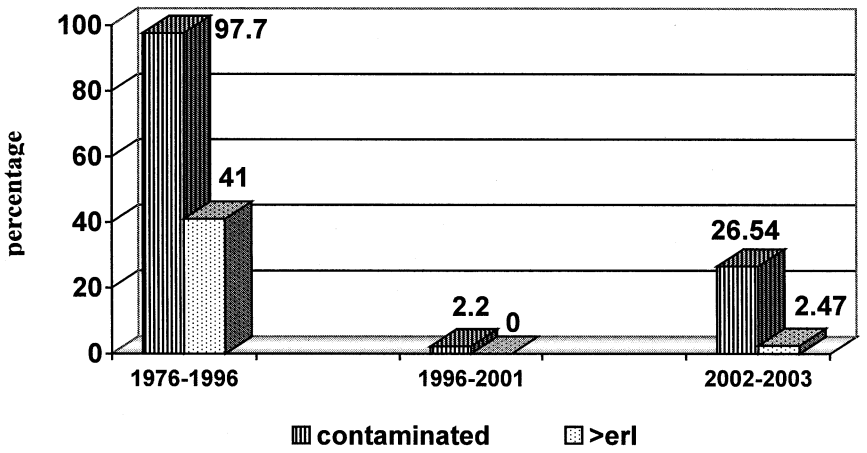

Figure 1. Milk samples contaminated with DDT residues in Punjab

form of p,p'- DDE, p,p'-DDD o,p'-DDT and p,p'-DDT. Nine of 36 samples from Hoshiarpur (25 per cent) contained total DDT residues with average and range of 0.01 mg kg^{-1} and BDL- 0.18 mg kg^{-1}. In only one sample, the residues exceeded the erl of 0.05 mg kg^{-1}. Eight milk samples from Jalandhar were found to be contaminated with residues of DDT and three exceeded the erl. DDT residues were present in the form of p,p'-DDE, p,p'-DDT and o,p'-DDE. Four samples from Ludhiana revealed the presence of DDT residues but none exceeded the permissible limit. DDT residues were present in the form of p,p'-DDE, and p,p'-DDT . However, nine samples from Mohali had DDT with an average of 0.01 mg kg^{-1} and a range of BDL – 0.21 mg kg^{-1} but only one exceeded the erl. Ten samples from Patiala had DDT residues at detectable levels but none exceeded the permissible limit. DDT residues were present in the form of p,p'-DDE, o,p'-DDT, p,p'-DDT and o,p'-DDE. Eight samples from Sangrur contained residues of DDT with only one sample exceeding the erl. Though the residues of DDT were detected in twelve samples from Bathinda but only one exceeded the permissible limit.

The residues of DDT in milk have shown declining trends in the present investigations. The studies conducted during 1976-1996 revealed high level of contamination of DDT residues (97.7 per cent) and 41 per cent of the samples exceeded the tolerance limit of 0.05 mg kg^{-1} (*Singh B, pers comm.*). During 1996-2001, out of the 92 samples analyzed in Punjab, only 2.2 per cent milk samples were found to be contaminated with DDT residues and none exceeded the erl. In the present study, 26.54 per cent milk samples were found to be contaminated with DDT and only 2.47 per cent samples had residues above the erl of 0.05 mg kg^{-1} for total DDT (Fig.1). The increased contamination may be due the large number collected from different agro-climatic regions of Punjab contrary to previous

studies where samples of milk were collected from Patiala and Ludhiana (Battu *et al.* 1996). DDT was banned for use in agriculture and its use has been restricted for public health programmes in India with effect from January 1996. Therefore, the possibility of aged residues of p,p'-DDE as a stable metabolite of DDT being excreted in milk samples cannot be over ruled. DDT is not absorbed by green plants. However, if the green fodder and wheat straw were contaminated by soil dust containing DDT residues then that could account for the contamination. The spraying of DDT for mosquito control in cattle sheds has also been reported to contribute significantly towards the contamination of dairy milk (Battu *et al.* 1989; Kapoor *et al.* 1980). Illegal diversion of DDT intended for anti-malaria use to agriculture could be one of the sources of detectable residues in agricultural commodities (Curtis and Lines 2000).

The milk supplied by MILKFED milk plants comes from far and near villages and hence considered to be representative of the large area. The milk has been found to contain on an average 0.01 mg kg^{-1} DDT residues in all the districts of Punjab. The average daily per capita consumption of milk in Punjab has been reported as 600 g and 875g for infant (Anonymous 2003). Thus the intake of DDT by a person through milk alone comes out to about 6 µg and 8.75µg that is 2 and 35 per cent of ADI for an adult weighing 60 kg and a three month old infant weighing 5 kg. However, if calculated on the highest level (0.21 mg kg^{-1}) reported in a sample collected from Mohali, this intake is seven times higher than the ADI for the same infant.

Thus these studies indicate that the contamination of milk with DDT residues has greatly come down which could be attributed mainly to its ban in agriculture and restricted use in public health. However, appropriate remedial measures need to be taken so as to get milk of acceptable quality.

Acknowledgments. We thank the Head, Department of Entomology, Punjab Agricultural University, Ludhiana, and various milk plants under MILKFED for providing the necessary facilities during the course of investigations. The financial assistance in the form of Novartis Crop Protection Fellowship provided to the author by Novartis is duly acknowledged.

REFERENCES

Anonymous (1999) Codex Maximum Residue Limits for Pesticides Joint FAO/WHO, CAC Vol XIII p 72. FAO, Rome.

Anonymous (2003) Statistical Abstract of Punjab 2003 Publication No. 879 Economic and Statistical Organization, Government of Punjab, Chandigarh.

Battu RS, Singh PP, Joia BS, Kalra RL (1989) Contamination of bovine milk from indoor use of DDT and HCH in malaria control programme. Sci Total Environ 86: 281-287.

Battu RS, Singh B, Kalra RL (1996) Seasonal variation in residues of DDT and HCH in dairy milk in Punjab, India. Pestic Res J 8: 32-37.

Battu RS, Singh B, Kang BK, Chahal KK (2001) Status of contamination of bovine milk with pesticide residues. In: Extended Abstr Int Conf on Pesticides, Environment and Food Security, pp 265-66. 19-23 November,2001, Society of Pesticide Science, IARI New Delhi, India.

Curtis CF, Lines JD (2000) Should DDT be banned by international treaty. Parasitol Today 16: 119-121.

Gupta A, Parihar NS, Singh V (1997) HCH and DDT residues in bovine milk and milk powder. Pestic Res J 9: 235-237.

Kalra RL, Kaur H, Sharma S, Kapoor SK, Chakraborty SS, Kshirasagar RB, Vaidya RC, Sagade RB, Shirolkar SB, Dikshith TSS, Raizada RB, Srivastava MK, Singh V, Nagarja KV, Appaiah KM, Srinivasa MA, Rani MU, Rao SNR, Toteja G S, Dasgupta J, Ghosh PK, Saxena BN (1999) DDT and HCH residues in dairy milk samples collected from different geographical regions of India: a multicentre study. Food Add and Contam16: 411-417.

Kapoor SK, Chawla RP, Kalra RL (1980) Contamination of bovine milk with DDT and HCH residues in relation to their usage in malaria control programme. J Environ Sci Hlth B15: 545-547.

Mathur BN, Thompkinson DK, Sabikhi L (2000) Milk- the nectar for living beings. Indian Dairyman 52: 11-19.